Journal of
Pulmonology and
Intensive Care

Original Article

DOI: 10.51271/JOPIC-0025

Diagnostic value of tumor M2-pyruvate kinase level in lung cancer and its relationship with tumor histological type

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Cite this article: Aslan Kara A, Gönenç Ortaköylü M, Uzun H. Diagnostic value of tumor M2-pyruvate kinase level in lung cancer and its relationship with tumor histological type. *J Pulmonol Intens Care*. 2024;2(1):6-10.

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Received: 28/10/2023 ◆ **Accepted**: 10/01/2024 ◆ **Published**: 13/02/2024

ABSTRACT

Aims: To investigate the diagnostic value of pyruvate kinase isoenzyme-M2 (M2-PK) levels and their relationship with tumor histological type in patients diagnosed with lung cancer.

Methods: In this study, 98 cases diagnosed with lung cancer (Study group) and 90 cases with lung cancer excluded (control group) were included. The study group consisted of people over the age of 18 who had been diagnosed with lung cancer and had not received any treatment for the tumor. The control group consisted of 45 people who had been diagnosed with any lung disease but did not have lung cancer, and 45 of them were completely healthy people. Those with benign lung disease apart from lung cancer were named as control group-1 and healthy control group was named as control group-2.

Results: M2-PK levels were measured and compared in the lung cancer group, control group-1 with non-lung cancer lung disease and healthy control group-2. M2-PK levels were found to be significantly higher in the lung cancer group than in the control group 1 and control group 2 (respectively $p<0.0001$, $p<0.0001$). When M2-PK levels were compared in all three groups, they were statistically significant in the lung cancer group ($p<0.001$). In our study, the diagnostic cut off value was found to be 8.9 IU/ml using ROC curve. At this cut-off value, plasma m2-pk level was calculated as 100% sensitivity and 97.8% specificity in showing lung cancer. When compared, there was no statistically significant difference between histopathological diagnoses, stage of the disease and M2-PK levels in the lung cancer group.

Conclusion: As a result of this study, it was concluded that tumor M2-PK can be used to distinguish lung cancers from other benign lung lesions and as a marker in patients with suspected lung cancer.

Keywords: Lung cancer, M2 pyruvate kinase, tumor marker

INTRODUCTION

According to the 2020 world cancer statistics, lung cancer ranks 2nd among the most common cancers in the world with 2.2 million (11.4%) new cases. With 1.8 million deaths per year, lung cancer ranks first among cancer-related deaths. WHO reports that lung cancer is the most common type of cancer in men worldwide, while it ranks 3rd in women. Therefore, lung cancer remains important with its frequency and mortality rate. In our country, the number of cancer patients who receive new diagnoses annually is also increasing in parallel with the increasing population. According to the published data, in 2020, 233.34 new cancer cases and 126.335 cancer-related deaths were reported in Turkiye, where the total population was 84.339.67. Lung cancer was reported to be the leading cause of cancer-related deaths in Turkiye (18%).[1,2]

Early diagnosis of lung cancer remains important because it is the leading cause of mortality and morbidity, and new biomarkers are needed as an important part of early diagnosis and prognosis.[3] Pyruvate Kinase is located in the glycolytic pathway. It is the enzyme that controls the production of nucleotide triphosphate, which has an important role in tumor metabolism and has 4 isoenzymes: L, R, M1, M2 types. Type M2 is released mainly in the lungs, kidneys, embryos. While M2pk, which is found in blood and other body fluids, is found in tetrameric form in normal cells, it is transformed from tetramer form to dimeric form in tumor cells, and its release increases in carcinogenesis for various reasons.[4-7] Measurement of tumor M2-PK; it can be very effective for detecting possible recurrence or metastasis and for monitoring the effects of treatment, along with providing useful supporting information in the diagnosis and diagnosis of various tumors. The level of tumor M2-PK can be measured in samples of blood and other body fluids.[8]

Various isoforms of purivate kinase, a glycolytic enzyme,

are released in many tissues. This enzyme, which is usually released from tissues in tetrameric form, has been shown to be synthesized at a high level in dimeric form (M2-PK) in tumor tissues. Many studies have shown that Tu M2-PK can be used as an important marker in the differentiation of benign and malignant cancer and prognosis in different types of cancer.

In our study, we aimed to investigate the diagnostic value of M2-PK level and its relationship with tumor histological type in patients diagnosed with lung cancer at S.B Yedikule Thoracic Diseases and Thoracic Surgery Training and Research Hospital.

METHODS

Ethical Consideration

The study was carried out with the permission of Yedikule Thoracic Diseases and Thoracic Surgery Training and Research Hospital. The study protocol was approved by the Ethics Committee of Yedikule Chest Diseases and Chest Surgery Training and Research Hospital. (Date: 04.02.2012, Decision No: 0006). We obtained an informed consent form from all patients for procedure. The study was conducted between 2012- 2013 and all procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

The study included 98 patients who were histopathologically diagnosed with lung cancer (study group) and control groups which 45 cases with non-malignant lung disease (control group-1) and 45 completely healthy individuals without any disease (control group-2). There were 10 Chronic obstructive lung diseases, 10 tuberculosis, 10 pneumonia, 5 idiopathic pulmonary fibrosis, 5 bronchiectasis and 5 pulmonary embolisms in the control group-1. Smoking, alcohol history, family history of lung cancer, diabetes mellitus, hypertension, and ischemic heart disease history were questioned among the variables evaluated.

Histopathological examinations were performed with surgical biopsies or bronchoscopic materials. These materials were examined in the pathology laboratory of our hospital and classified as small cell, lung cancer and non-small cell lung cancer. Patients over 18 years of age, who had been diagnosed with lung cancer histopathologically, who had not previously been diagnosed with any malignancy, and who had not received any treatment for the identified tumor were included in the study.

Determination of Plasma M2-PK Levels

After obtaining the informed consent form from the patients, a plasma sample with 5 cc of EDTA was taken and these samples were quickly frozen and stored in this way (at -80 degrees) until laboratory analysis was performed. Commercial kit based on the sandwich ELISA principle (ScheBo Biotech Ag, Tumor M2-PK ELISA kit, Germany) was used to determine plasma M2-PK levels. The intra and inter-assay variation coefficients were 5.2% (n=25) and 6.3% (n=25) respectively.

Statistical Analysis

Statistical analyses were performed using SPSS (Statistical Package for Social Sciences) for Windows 16 Release 16.01 package program (SPSS, Inc. Chicago, IL, USA). Only the MedCalc program was used in the ROC Analysis, which determines the cut off value and minimizes the margin of error. $p<0.05$ was considered significant.

RESULTS

The ages of the patients with lung cancer were 59.37±9.16, the ages of the control group-1 were 60.62±13.31, the ages of the control-2 were 60.22±11.04 When the correlation analysis was performed between age and M2-PK values in the study group, control group-1 and control-group-2, no significant correlation was found between age and M2-PK levels in any of the groups. (respectively, r=-0.1973, p=0.0515; r=0.0324, p=0.8323; r=- 0.0971, p=0.5306) There was a history of co-morbidity (1 coronary artery disease, 15 Hypertension, 11 Diabetes mellitus, 1 myasthenia graves, 1 chron disease) in the study group. History of co-morbidity in control group -1 (8 DM, 7 HT, 2 CHF, 1 IHD, 2 Rheumatoid Arthritis, 1 Guillain-Barre Syndrome). Mean plasma tumor M2-PK levels were 17.17± 7.75 IU/ml in the patient group with lung cancer, 4.53±2.15 IU/ml in the control group and 4.08±2.87 IU/ml in the healthy group

M2-PK levels of the groups M2-PK levels were significantly higher in the lung cancer group than in the control group-1 ($p<0.001$) and control-group-2 ($p=0.0001$). When M2-PK levels were compared in all three groups, they were statistically significant in the lung cancer group ($p<0.001$). Also student t test analyses results suggest that M2pk levels in lung cancer group were statistically significant compared with control group and healthy control group M2-PK levels (respectively, $p<0.0001$, $p<0.0001$) (**Table 1**, **Figure 1**).

Table 1. Comparison of M2pk values between 3 groups

	Patient group	Control group	Healthy control group	p
M2PK (U/ml)	17.17±7.75	4.53±2.15	4.08±2.87	<0.001
Mean±SD	9.68±45.43	0.5±10.8	0.07±17.17	<0.001

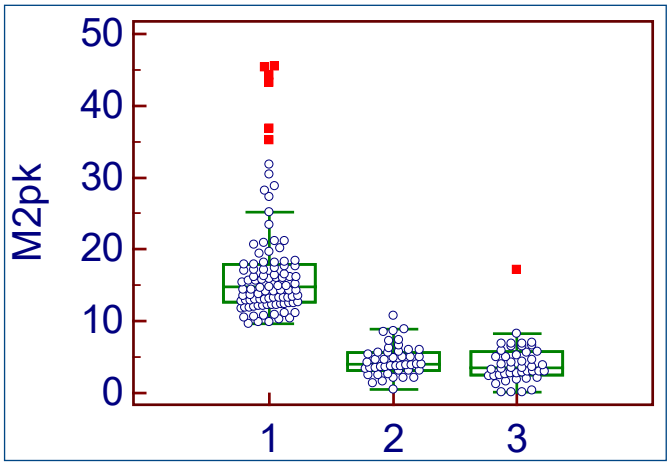

Figure 1. Comparison of M2pk values between 3 groups (1: Patient Group, 2: Control Group, 3: Healthy Control Group.)

M2-PK levels of patients in the lung cancer group were compared according to the demographic characteristics of the patients. There was no statistically significant difference between the M2-PK levels of the lung cancer group and the history of smoking, alcohol use, co-morbidities, and family history of malignancy. When compared in terms of sex, M2-PK levels were found to be statistically significantly higher in men (**Table 2**).

Table 2. Comparison of M2-PK values according to demographic characteristics in the lung cancer group

Study group		n	%	M2PK (U/ml) Mean±SD	p value
Gender	Female Male	10 88	10.20 89.79	13.56±2.03 17.58±8.06	<0.001
Cigarette	Yes None	89 9	90.81 9.18	17.38±8.04 15.01±3.4	0.111
Alcohol	Yes None	23 75	23.43 76.53	18.91±10.4 16.63±6.74	0.332
Comorbidity	Yes None	22 76	22.44 77.55	16.94±7.47 17.23±7.88	0.873
Family cancer history	Yes None	17 81	17.34 82.65	16.91±8.25 17.22±7.70	0.887

In the lung cancer group, there was no statistically significant difference between the histopathological diagnosis, stage of the disease and M2-PK levels in the lung cancer group (**Table 3**).

Table 3. Comparison of M2-PK levels according to histopathological diagnosis and cancer stages in lung cancer group

	M2PK(U/ml) Mean±SD	p
Diagnosis		0.298
Small cell ca (n=14)	19.8±10.25	
Non-small cell ca (n=84)	16.73±7.24	
Stage		
1 (n=3) 2A (n=8)	16.61±3.92 18.99±10.02	0.584
2B (n=5) 3A (n=18)	13.79±3.98 16.15±6.52	0.337
3B (n=21) 4 (n=43)	16.74±7.59 17.89±8.49	0.585

ROC analysis was performed to investigate the diagnostic value of the M2-PK marker in the diagnosis of lung cancer. The cut-off value of M2-PK level was calculated as 8.9 IU/Ml. According to these values, the sensitivity of M2-PK marker in the diagnosis of lung cancer was calculated as 100%, specificity: 97.8%, false positive value as 2.2%, false negativity value as 0%, and accuracy as 98%. Area under the ROC curve (AUC) 0,991 (**Figure 2**).

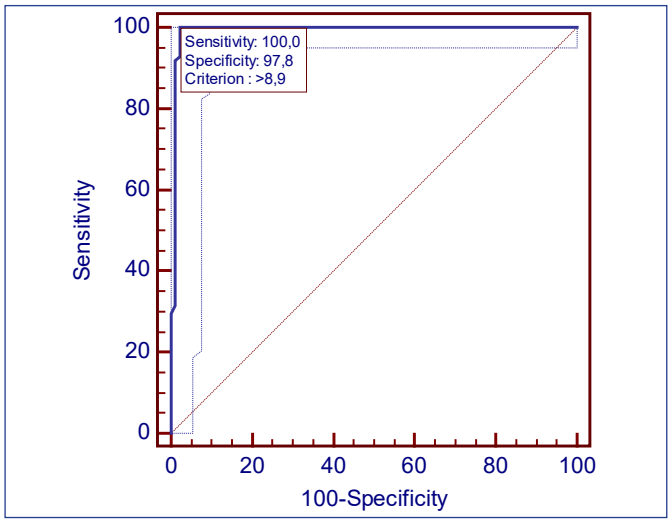

Figure 2. The value of the m2pk marker in the blood, ROC analyses

As a result of this analysis, the area under the ROC curve was calculated as 0.991 and obtained as p<0.0001. This value shows that the blood levels of the M2PK marker are 99.1% stronger in diagnosing AC cancer and can distinguish between patient with lung cancer and healthy individuals as 99.1%.

DISCUSSION

While lung cancer was a rare disease at the beginning of the 20th century, its frequency gradually increased in parallel with the increase in smoking habit and became one of the most common cancers in the world. It ranks first in cancer-related deaths all over the world.[9,10]

In our study, M2-PK levels were evaluated as tumor markers in patients with lung cancer. When the lung cancer group and the control group with non-lung cancer lung disease were compared, the tumor M2-PK level was found to be significantly higher in the lung cancer group (p<0.001). There was no significant difference between tumor M2-PK levels in terms of smoking history, alcohol use history, additional disease history, family history of malignancy.

Early diagnosis and treatment are the most effective way to prevent and reduce mortality, which numerous studies and prevention data have confirmed. Imaging and bronchoscopy have an important value in the diagnosis of lung cancer.

Numerous studies and prevention data have confirmed that early diagnosis and treatment are the most effective way to prevent and reduce mortality. Imaging and bronchoscopy have an important value in diagnosing lung cancer. In 2013, the U.S. Preventive Services Task Force (USPSTF) recommended annual lung cancer screening with low-dose computed tomography (DDCT) in adults 55 to 80 years of age who have a history of smoking 30 packs of cigarettes per year and who are currently smokers or have quit smoking in the past 15 years.[11]

On the other hand, a study was conducted in active smokers and former smokers investigating the risk of radiation-induced lung cancer associated with DDCT for annual screening and the baseline risk that the potential benefits of this screening should overcome. Given the estimated upper limit increase of 5.5% in the lung cancer risk attributable to annual CT-related radiation exposure, it was also emphasized that there would have to be a mortality benefit of more than 5% to outweigh the potential radiation risk.[12]

These are widely used clinical medical methods, but they are not easy to use in large-scale screenings. The level of tumor markers has a good correlation with the formation of tumors. Therefore, the describe of tumor markers, which is a noninvasive method that can be used for early diagnosis and prognosis, has recently become the focus of attention.

M2-PK levels were also found to be high in benign diseases. In the study conducted by Oremek et al.[13] tumor M2-PK levels were found to be high in patients with rheumatoid disease, seronegative spondylarthritis and patients with collagen tissue disorders. One study showed that a single change in the isoform of the glycolytic enzyme pyruvate kinase in tumor formation is necessary for the shift of cellular metabolism to aerobic glycolysis.[14]

It has been reported that this enzyme is high in the diagnosis and prognosis of periampullary pancreatic cancer and in the follow-up of cervical cancers and can be used as a tumor marker. A study demonstrating the role of tumor M2-PK enzyme in lung cancers has been conducted in the literature and it has been thought that this marker can be used to distinguish between benign and malignant lung lesions.[15-18] Christofk et al.[14] show that tumor M2PK is a non-invasive test to diagnose colorectal cancer and adenomatous polyps.

In a study investigating the relationship between pyruvate kinase M2 (PKM2) expression and prognosis in 86 hepatocellular cancer (HCC) patients, it was shown that M2PK expression level was significantly higher in HCC tissues than in healthy tissues. And it was concluded that M2PK expression can be used as a prognostic marker.[19]

In addition, there are studies for the sensitivity comparisons of tumor M2-PK with some markers used in tumor diagnosis and follow-up. Maurizio et al.[17] compared tumor M2-PK with CA 19-9 in a total of 265 cases with acute, chronic pancreatic cancer, benign pancreatic and control group. As a result, they reported that tumor M2-PK level could be used as a metabolic marker, but that it would be more meaningful to use it with CA 19-9 tumor marker.

In the study in which Li Li et al.[20] analyzed the diagnostic and prognostic values of serum TuM2-PK, NSE and ProGRP values in small cell lung cancer, it was shown that M2-PK, NSE and ProGRP levels were higher than the control groups with benign lung disease and healthy control groups. Tu M2 PK sensitivity was found to be 82.35% and serum Tu M2 PK level may be an effective marker of small cell lung cancer and an independent prognostic factor for shorter survival.

Chunhua et al.[21] looked at serum tu M2PK levels in patients with early-stage NSCLC and found it to be higher in patients with NSCLC than in the control group with healthy and benign lung disease (sensitivity 71.6%, specificity 98%), and high serum TUM2-PK level of early-stage NSCLC may be a potential biomarker for the diagnosis and prognosis of early-stage NSCLC patients. A Chinese meta-analysis by Juncai Liu et al.[22] concluded that serum tumor M2PK may be a potential biomarker in the diagnosis of NSCLC.

In our study, M2-PK levels were evaluated as tumor markers in patients with lung cancer. The study included 98 patients with histopathologically defined lung cancer, 45 patients with non-malignant lung disease and 45 healthy individuals. When the tumor M2-PK levels of the cases were compared, the mean value was found to be significantly higher in patients with lung than in both control groups. ($p<0.001$) When the lung cancer group and the control group with non-lung cancer lung disease were compared, the tumor M2-PK level was found to be significantly higher in the lung cancer group ($p<0.0001$). There was no significant difference between tumor M2-PK levels in terms of smoking history, alcohol use history, additional disease history, family history of malignancy.

There was no significant difference between tumor M2-PK plasma levels and histopathological types of cancer (small cell and non-small cell lung cancer) in patients with lung cancer. When patients with lung cancer were compared in terms of sex, plasma tumor M2-PK levels were significantly higher in both sexes than in control groups, but they were higher in men than in women, and the difference between them was statistically significant (male: 17.58±8.06; female: 13.56±2.037; $p=0.0005$).

In our study, the diagnostic cut-off value was found to be 8.9 IU/ml using ROC curve. At this cut-off value, the sensitivity of tumor M2-PK marker in showing lung cancer was calculated as 100% and the specificity was calculated as 97.8%.

Limitations

The limitations of the study are limited number of patients, 65% of patients have advanced-stage lung cancer, the results reflect only the population from Turkiye, the number of female patients is low, more studies are needed to reveal the role of tumor M2-PK in carcinogenesis.

CONCLUSION

As a result of this study, it was concluded that tumor M2-PK can be used as a marker in the differentiation of lung cancers from other benign pulmonary lesions as well as a screening marker in patients with suspected lung cancer Because of its low cost and high sensitivity and specificity, M2-PK can be used as a screening test for lung cancer. Comparative studies with other screening tests are needed in a larger population.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ethics Committee of Yedikule Chest Diseases and Chest Surgery Training and Research Hospital. (Date: 04.02.2012, Decision No: 0006).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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